Review

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The treatment of black stain associated with of iron metabolism disorders with lactoferrin: a litterature search and two case studies

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Abstract

Among the various pathologies of the oral cavity, the formation of "unsightly black spots" on the surface of the tooth, universally known as Black Stain (BS) has recently been acquiring more interest. Usually BS is typically found in individuals in prepubertal age, even though it has been identified in adults associated with microbial exchange and / or with iron metabolism disorders.

Microbial exchange concerns the possible exchange of bacteria between family members which can take place directly, through effusions, or indirectly, through brushes, cutlery or glasses. For this reason, it is recommended that toothbrushes of family members not be left damp and in contact with each other. The bathroom, being a warm-humid environment, is in fact an optimal habitat for microbial proliferation.

Of specific importance in BS is the accumulation of iron in tissues and secretions which, together with chromogenic bacteria, are the primary cause of this pathology. In fact, among the metabolic products synthesized by bacteria in the oral cavity, hydrogen sulfide is of considerable interest, since upon reacting with iron available in saliva, in pathological conditions (iron metabolism disorders), it forms black precipitates consisting of ferric sulfide. These precipitates bind to the surface of the teeth, tending to form a stria that usually follows the contour of the gingiva, with an unsightly and variable chromatic intensity. In physiological situations, iron homeostasis is defined as the state of equilibrium between iron present in tissues and in secretions and that which is present in the circulation. Instead, in pathological conditions, defined as iron metabolism disorders, there is an accumulation of iron in tissues and secretions and a lack of it in the circulation.

It is also important to remember that subjects affected by BS are more protected from carious processes than healthy subjects, probably due to a significant predominance of chromogenic bacteria compared to those responsible for caries. It should also be remembered that in young subjects BS tends to regress with pubertal development and the transition to adult life.

In any case, using common professional hygiene procedures, it is possible to remove BS as well as plaque and tartar deposits. In particular, with ultrasonic scalers, polishing pastes and powders carried by air and water jets, the surfaces of the teeth can be restored to their natural healthy state.

All the techniques for removing the precipitates, are not enough however, to fix and permanently eradicate their appearance, as these precipitates last only for short periods and recur very frequently. Due to the frequent recurrences, new oral microbiota control therapies are emerging; among these the use of lactoferrin (Lf) in the dental field and particularly in the treatment of BS appears to be very promising. Taken togheter, here the effect of Lf in subjects affected by BS has been investigated. ***Clin Ter 2019; 170(5):e373-381. doi: 10.7417/CT.2019.2163***

Key words: *lactoferrin, black stain, iron metabolism, oral disorders, saliva*

A General background

Lactoferrin

Lactoferrin (Lf) is a monomeric glycoprotein with a molecular weight of about 80 KDa, indicated as a red protein due to its characteristic color caused by its completely saturated iron shape. In fact, this glycoprotein has the ability to chelate two ferric ions (Fe^{3+}) per molecule. The isoelectric point (P.I.) of Lf is between 8.4 and 9.0, a higher value than that observed for the other transferrins (P.I. 5.4-5.9) (1-4). Lf belongs to the transferrin family (Tfs) proteins which chelate two ferric ions per molecule, including ovotransferrin (oTf) and serum Tf (sTf) (5).

Chromosomal analysis was able to identify human and murine Lf genes on chromosomes 3 and 9 (6-8) respectively and caprine and bovine Lf genes on chromosome 22 (9,10). The bovine Lf gene is located in a DNA fragment of about 34.5 kb and is organized in 17 exons and 16 introns (11). The leading peptide consists of 19 amino acid residues encoded by the last 14 codons of the first exon and the first 5 of the second exon. Human Lf consists of a single polypeptide chain of 692 amino acids, organized in two globular lobes, corresponding respectively to the amino acid residues 1-333

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(lobe N) and 345-692 (lobe C), joined by an α-helix comprising the amino acid residues 334-344 (12).

Each of the two lobes of Lf consists of two domains (N1 and N2 for the N-Lobe and C1 and C2 for the C-Lobe) encoded by specific exons. For the N-lobe, exons 2-4 code for the first domain and exons 4-7 for the second domain. Similarly, for the C-lobe exons 9-12 encode for the C1 domain and exons 12-15 for the C2 domain (13). Each lobe has a single Fe^{3+} binding site located in a pocket between the two domains (14). In each site, the binding of Fe^{3+} occurs through the combined action of 4 identical amino acid residues for each lobe (Asp58, Tyr93, Tyr193 and His254 in the N-lobe; Asp396, Tyr434, Tyr527 and His596 in the C-lobe). The binding with Fe^{3+} is stabilized by the bicarbonate ion (HCO_3^-). In fact, through the neutralization of the positive charge of the arginine residue (Arg121 in the N-lobe) and the associated α-helix, the HCO_3 anion stabilizes the binding of Fe^{3+} to the four ligands (15). Lf is also able to chelate two Cu^{2+} ions in the same binding sites of Fe^{3+}, and four Zn^{2+} ions, in sites different from those chelating iron (16,17). Furthermore, it should be emphasized that apo-Lf means that the protein is iron-free and takes on an open conformation, whereas holo-Lf, the protein completely saturated in Fe^{3+}, takes on a closed conformation (15,18).

The amino acid sequence of human Lf (hLf) shows a high homology (~ 60%) with human sTf: the two globular lobes have 125 amino acid residues in common (homology of ~ 40%) and a very similar tertiary structure, probably as result of the duplication of an ancestral gene (12,19).

The following is the number of amino acids that make up the various Lfs:
- Human Lf: 692 amino acids (20);
- Bovine Lf: 687 amino acids (21);
- Murine Lf: 688 amino acids (22);
- PorcineLf: 685 amino acids (23);
- Caprine Lf: 690 amino acids (9);
- Bubaline Lf: 689 amino acids (24).

Moreover, hLf has a high sequence homology with bovine Lf (bLf) (25). The caprine Lf and the bLf present a homology of 94.9% (21), while the caprine Lf has a homology degree of 76.1% with the hLf (9), 78.2% with the porcine Lf (23), 69.5% with murine Lf (22) and 91.1% with Lf extracted from buffalo milk (24).

More than 50% of eukaryotic proteins are glycosylated and this glycosylation plays an important role in the biological functions of proteins. Lf is no exception; therefore, even if the sequence homology between the various Lf is high, the glycan profiles are different. In fact, hLf has three potential glycosylation sites in correspondence with asparagine (Asn) 138, 479 and 624, while bLf has five at Asn233, 281, 368, 476 and 545 (26). Of all these glycosylation sites, only two are commonly glycosylated in hLf (Asn138 and Asn479) and four in bLf (Asn233, Asn368, Asn476 and Asn545) (27).

These differences are generally associated with different functions. In particular, glycans influence protein folding, conformation, solubility, immunogenicity, antigenicity (28) and resistance to proteolysis (17,29).

Lf was identified, initially, in human and bovine milk (30,31) and, subsequently, in the milk of other mammals (goats, pigs, horses and mice) (32). Today we know that Lf is present in small quantities even in human secretions such as saliva, tears, bile, seminal fluid and pancreatic juice (33.34). The concentration of Lf varies widely in different species, with it being higher in human milk. Swine and murine species have a relatively high content of this protein in milk compared to the bovine species and other ruminants such as goats (35) and sheep (36). In all species, however, the concentration of Lf is higher in the colostrum and can increase considerably in case of infections, thanks to the neutrophils which contain it in their granules. Lf is also present in the plasma, although at very low concentrations (37) which, also in this case, may increase during infections thanks to recruitment of neutrophils (38,39).

Functions of lactoferrin

Both hLf and bLf are multifunctional proteins that have a wide spectrum of biological activities. It is interesting to note that hLf in breast milk plays a primary role in newborns where it reaches the intact intestine due to a low acidity of gastric juice. Therefore, it is resistant to proteolysis and is active at a neutral pH and like bLf, performs its role in the intestinal lumen starting from the duodenal area, allowing:
- absorption of Fe (40.41);
- modulation of hematopoiesis (42);
- antimicrobial activity (43);
- immunostimulatory action (44);
- activity inhibiting the release of histamine from mast cells (34);
- modulation of Fe homeostasis (41, 45-48);
- anti-inflammatory activity (47,49).

Furthermore, Lf reduces the development of tumor cells in patients with leukemia and inhibits the formation of metastases in breast cancer (50,51).

These results could suggest an important involvement of this protein in protecting against the development of tumors, providing new approaches to anticancer therapies.

The most commonly known function of Lf is its marked antimicrobial activity against many pathogenic species (43). The antimicrobial effect of Lf is attributed to both its ability to bind Fe^{3+} and its ability to interact with microbial surfaces (13,43).

In particular, Lf performs a bacteriostatic action through the sequestration of Fe^{3+}, an essential element for microbial growth.

It has also been suggested that iron sequestration by Lf has a protective effect against *Mycobacterium tuberculosis* infections (52). Likewise, it plays a crucial role in the prevention of chronic lung infections associated with *Pseudomonas aeruginosa*, where Lf inhibits biofilm synthesis (53). However, in some cases, these activities are only temporary. In fact, bacteria respond to the absence of iron through the production of molecules known as siderophores, capable of chelating one ferric ion per molecule, thus competing with the Lf for the acquisition of this ion (54). In addition, some bacterial species belonging to the Neisseriaceae family, such as *Neisseria meningitidis* (55), have adapted to the restrictive conditions imposed by the absence of iron, synthesizing specific receptors able to bind to Lf and acquire this element directly from the protein (56).

In any case, the sequestration of Fe^{3+} by Lf delays the growth of some pathogens, while it favors the duplication

of commensals such as lactobacilli that do not require this element for growth (57).

Lf is also able to perform a bactericidal action through interaction with microbial surfaces (43,58). Through this interaction, Lf is able to damage the membrane of Gram-negative bacteria by binding to lipopolysaccharide (LPS), porines and other surface molecules (43,59). The binding and successive release of LPS causes an increase in membrane permeability, thus making the bacterium more sensitive to the action of antibiotics (60) or inducing lysis. However, it has also been demonstrated that the presence of Ca^{2+} and Mg^{2+} ions stabilizes bacterial membranes, inhibiting the release of LPS and the consequent microbial lysis by Lf (47). Furthermore, the Fe^{3+} chelating activity of the Lf is temperature and pH dependent (61). Finally, the digestion of bLf and hLf with pepsin leads to the formation of a series of cationic peptides, called "lactoferricins", with high bactericidal activity (47,62). In fact, these peptides, which are not able to chelate the ferric ion, have, however, the ability to interact with LPS by lysing bacteria.

Moreover, it has been established that Lfs, in addition to having an antibacterial action, are able to exert an antifungal, antiparasitic and antiviral action. It is known, in fact, that Lfs and their peptides act efficiently against Candida spp. infections. (63,64). Moreover, Lfs have a confirmed antiparasitic activity exercised through different mechanisms (65,66).

Lfs also exert an antiviral action. Infact, it has been shown that this protein is active against hepatitis C virus (HCV) (67), Polyomavirus, Rotavirus, Herpes simplex virus (HSV), Cytomegalovirus and HIV (68 and references included). The antiviral activity carried out by Lf is probably due to a double effect:

1. the ability of Lf to bind to glycosaminoglycans present on the membranes of eukaryotic cells, preventing the penetration of viral particles and therefore infection;
2. the ability of Lf to chelate Fe^{3+} by sequestering it from viral enzymes that need this metal as a cofactor for their replication.

In addition to antibacterial, antifungal, antiparasitic and antiviral action, Lf has strong anti-inflammatory activity and is considered a natural antioxidant, reducing the damage caused by free radicals (69). Particulary by sequestering Fe^{3+}, Lf inhibits the formation of reactive oxygen species (43, 70).

Moreover, Lf plays an important role in the immune response, modulating the anti-inflammatory processes by regulating the proliferation and differentiation of cells of the immune system (71). Some anti-inflammatory functions may be due to the ability of Lf to bind LPS, decreasing its toxicity (69,72,73). The high concentration of Lf in breast milk, the high affinity of this protein for Fe^{3+} and the great bioavailability of Fe^{3+} has also suggested a fundamental role of Lf in the regulation of iron homeostasis and intestinal absorption of this element in newborns and adults (41, 74). In fact, on the surface of the intestinal mucosa of many species, specific receptors for Lf (75) have been identified that become part of mechanisms that look after the duodenal absorption of iron (76). Furthermore, on the apical part of enterocytes, Fe^{3+} is reduced by duodenal cytochrome B (DcytB), in order to then be transported inside these cells by a transporter molecule (DMT-1). In the cytoplasm iron is then stored in the ferritin. The transport of iron from cells to the circulation is then guaranteed by ferroportin (Fpn) after having been oxidized by hephaestine (Heph). In the blood, Fe^{3+} is therefore available for binding to apo-transferrin which will transport it to various tissues (77).

Oral cavity and lactoferrin

The human body is composed of 10^{13} eukaryotic cells colonized by 10^{14} commensal (non-pathogenic) bacteria which make up the microbiota. All mucous membranes, including the oral mucosa, are characterized by their own microbiota, which plays a protective role against attack by pathogenic microorganisms. Oral pathologies can often arise following infection due to endogenous pathogens. For example, the fungus *Candida albicans*, despite being a component of the oral microbiota (78), can cause infections in subjects suffering from periodontitis as well as in immunocompromised subjects such as the elderly, diabetics, those suffering from HIV and cancer, subjects who have undergone prolonged antibiotic or anticancer therapies. An important line of defense against candidiasis is the oral microbiota and some anti-fungal components of saliva. Obviously, the cases of oral pathologies due to *Candida albicans* represent a microbial dysbiosis (alteration of the balance of microbial species that exist in the microbiota) as it is for disorders of the salivary components and damage to the epithelia. The oral cavity is colonized by a high number of bacteria ($5x10^8$ / 1 ml of saliva and 10^{10}-10^{11}/1 g of plaque) which can be present transiently (see *Helicobacter pylori*) or constantly (79). The high number of microorganisms present in the oral cavity is due to the colonization of endogenous bacteria, as well as easy accessibility of the district to microorganisms from the external environment, and to the presence of not only the mucosa, but also the abiotic surfaces (such as teeth) that make the site of microbial colonization important (80). The teeth, in fact, are covered by a layer of salivary proteins and glycoproteins (such as lysozyme, lactoferrin, lactoperoxidase, secretory immunoglobulin) which form a film, which after brushing of teeth adheres afresh and rapidly. This film interacts with microorganisms and favors their colonization in the oral cavity (*Streptococcus mutans, S. salivarius, S. mitis, S. oralis, S. sanguinis and S. gordonii,* Spirilli, Lactobacilli, Actinomiceti, etc.) thus forming dental plaque.

The most frequently isolated microorganisms from the oral microbiota of healthy subjects are: *Streptococcus, Actinomyces, Veillonella parvula and V. alcalescens, Fusobacterium, Porphiromonas, Prevotella, Treponema, Actinobacillus / Aggregatibacter actinomycetemcomitans, Nisseria flavescens, Haemophilus, Fusobacterium nucleatum, Eubacteria, Lactobacillus, Treponema denticola, T. macrodentium, T. orale, Capnocytophaga, Eikenella, Leptotrichia, Propionibacterium* (80,81).

Among these, the most important Gram-negative bacteria, such as *Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans, Treponema denticola, T. macrodentium, Neisseria flavescens, Veillonella parvula* and *V. alcalescens*, are facultative intracellular anaerobes, that migrate from the supragingival plaque to the subgingival plaque causing pa-

thological phenomena against the periodontium (80-82).

Generally, bacterial plaque adhers to the surface of the tooth, multiplies and forms the so-called biofilm. Biofilm formation occurs by adhesion, first reversibly (through electrostatic interactions, Coulomb and van der Waals forces) and then irreversibly; bacteria finally, becoming covered by an extracellular polysaccharide synthesized by themself (83,84). The mass of bacteria and exopolysaccharides constitute the biofilm.

Microbial adhesion, and subsequent biofilm formation, takes place not only on the tooth surface but also on resins and dental materials, on prosthetic and orthodontic appliances as well as on epithelial cells at the level of the oral mucosa (81,83,84).

Recently it has emerged that some oral bacteria are associated with severe pathologies such as endocarditis due to *Streptococcus mutans*, a facultative intracellular bacterium (85). Furthermore, periodontal disease is also associated with cardiovascular disease (86), cerebrovascular strokes (87) and a high risk of pre-term birth (88). An association between diabetes mellitus and periodontal disease has also been demonstrated. This correlation is bidirectional, with periodontitis having a negative effect on diabetes mellitus and vice versa (89). Furthermore, even if the association between periodontal diseases and inflammation is not well known, it is well-defined that inflammation causes an increase in the severity of many diseases.

Saliva analysis is considered a non-invasive test to monitor health status and is used to prevent pathologies in subjects (90). Obviously, it is necessary to carefully follow the specific guidelines for sample collection in order to minimize any errors (91). In particular, food and beverages must not be consumed at least 2 hours before sampling, and alcoholic beverages must not be consumed 24 hours before sampling, in order to avoid unreliability and repeatability errors in the tests and the relative results (92).

A healthy person produces 1000-1500 ml of saliva every day which is swallowed, and its components absorbed in the intestine.

Saliva, produced by the exocrine glands (such as the salivary, sublingual and submaxillary glands), is composed of peptides, inorganic (Fe^{3+}, Mg^{2+}, Na^{+}, K^{+}, Ca^{2+}, Cl^{-}, $HPO4^{2-}$ and HCO^{3-}) and organic compounds, various enzymes and hormones (cortisol, testosterone, dehydroepiandrosterone, progesterone, aldosterone and estrogen) (90,91,93). Furthermore, saliva is rich in antibacterial substances such as Lf, lysozyme, mucins (MG1 and MG2), immunoglobulins (IgA, IgM, IgG), alpha-amylase and organic compounds such as albumin, urea, uric acids, lactates and creatinine (93).

Lf is the most important factor in the natural immunity of saliva (18). Salivary Lf, in addition to having antimicrobial activity thanks to its ability to bind Fe^{3+}, also possesses powerful anti-inflammatory activity, in vitro (94) and in vivo (41,48,95-98), as already mentioned.

Saliva contains about 20 µg / ml of Lf (99). The levels of Lf are, however, altered in subjects suffering from oral pathologies (99-101). There is a decrease in the concentration of Lf in infectious diseases of the oral cavity (97) associated with pathological inflammatory processes. This destructive pathological inflammation is due to the lack of Lf in saliva and other factors such as free and available Fe^{3+} overload (about 100 µM) which stimulates microbial multiplication, ROS synthesis, inflammatory processes, pigment formation and the onset of Black Stain (BS) (102). In physiological situations, saliva contains an intra- and extra-cellular free iron concentration that fluctuates between 0.1 µM and 1.0 µM (depending on whether the analysis is performed before or after meals), while, in pathological situations it reaches significantly higher concentrations (15). The pathological increase in iron concentration is a consequence of disorders in iron homeostasis (17): iron overload in tissues and secretions, and deficiency of circulating iron (iron deficiency anemia).

As previously mentioned, BS represents a condition of the oral cavity characterized by the formation of a stria, which usually follows the contour of the gingiva, with variable chromatic intensity classified according to Gasparetto (103). This imperfection is, however, correlated with a significant resistance to the cariogenic process. In fact, subjects suffering from BS are generally "protected" from caries and, often, also from gingivitis. It seems that the bacteria involved in the formation of BS do not allow the colonization of bacterial species associated with some pathological processes affecting the hard and soft tissues of the oral cavity, such as caries and gum inflammatory processes (104). The BS phenomenon is due to a chemical reaction between H_2S, a metabolite synthesized by anaerobic bacteria, and the ferric ion present at high concentration in iron metabolism disorders. It should be noted that when there is an overload of iron in the tissues and in the secretions, in the circulation there is a deficiency of this element, which leads to anemia due to iron deficiency or, if related to an inflammation, anemia of inflammation. Therefore, knowing that Lf is a protein capable of performing an iron-dependent antimicrobial action based on the ability to chelate two ferric ions per molecule and an iron-independent action based on the binding of this cationic protein to the anionic surface components of the bacterium, it is conceivable that these two conditions could be favorable to the inhibition of the formation of BS.

The presentations of clinical cases

An observational clinical study

In the first observational clinical study, the efficacy of Lf was demonstrated in subjects suffering from the unaesthetic condition (1). The protocol included oral administration of Lf (Forhans Gengi-For® orosoluble tablets containing 50mg Lf + 50mg D-Biotin) twice a day after proper and accurate oral hygiene. This treatment was performed after professional and home hygiene practices (Fig. 1).

The encouraging results obtained confirmed our hypothesis, since Lf with respect to BS could perform two functions simultaneously: an antibacterial activity against the anaerobic microorganisms (responsible for BS) and an iron sequestration which makes it no longer available for the formation of ferric sulfide. Therefore, based on this data we improved the experimental protocol as follows:

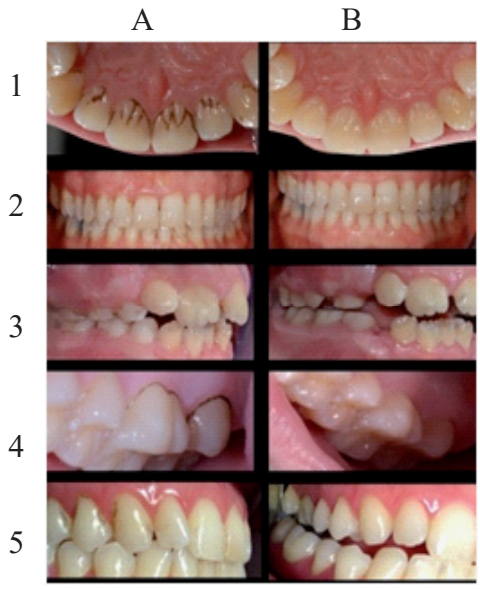

Fig. 1. Effect on Black Stain after 90 days of treatment with Forhans Gengi-For® (B) in 5 patients previously treated with professional hygiene sessions (A) (1).

Forhans Gengi-For® protocol in the eradication of Black Stain in adult subjects

- Check the familiarity of the subject for Black Stain
- Identify any subjects of the same family with Black Stain
- Enrollment of the subject and affected family members
- Select the degree of Black Stain according to the Gasparetto classification (103)
- Photograph the teeth affected by Black Stain before starting any type of treatment
- Teach proper home dental hygiene techniques
- Perform professional dental cleaning
- Carry out Air flow treatment with accurate polishing
- Prescribe 2 orosoluble tablets per day of Forhans Gengi-For®
  - to be taken after oral hygiene
  - to be dissolved completely and slowly in the mouth, spreading the solute on the surface of the teeth and in the interproximal spaces with the tongue
- Perform the first check-up at 2 months, documenting the possible effect of the administration of Forhans Gengi-For® by taking photos of the treated teeth and checking the patient's compliance.
- Hygiene rules to be respected:
  - use a new traditional or electric / sonic toothbrush
  - let the brush dry after use and do not insert it in the brush cover
  - get rid of toothbrush holders
  - prescribe a toothpaste Forhans Scudo Naturale containing lactoferrin
- Check-up by the dental hygienist after 2 and 6 months:
  - 2 months: photographic documentation of the effectiveness of the treatment
  - 6 months: based on the results obtained and evaluating the case based on the Gasparetto classification, (103), decide whether to carry out intervals of Lf or switch to a tablet every night or proceed only with Forhans Scudo Naturale, the toothpaste containing lactoferrin.

This new protocol has been applied in four other patients and the encouraging results obtained, are shown in Fig. 2.

Administration of Forhans Gengi-For® in uncooperative children

In the case of small children, the Forhans Gengi-For tablet should be crushed and mixed with a few drops of water to form a thick paste

Two times a day, after each meal and careful oral hygiene, the mother will have to apply the finger paste on teeth, with closer attention paid to the parts that show greater pigmentation.

A case report

During pregnancy, various changes occur in the woman, where important hormonal variations are observed with significant consequences during gestation. Furthermore, with regards to demand for iron, it increases from 1-2 mg / day in the first trimester to 4 mg / day in the second trimester and to 8 mg / day in the third trimester. It should also be remembered that serum IL-6 levels increase in physiological pregnancies and to a greater extent in pathological ones. Considering that pregnancy, childbirth and breastfeeding involve a total consumption of 1500 mg of iron, iron deficiency anemia is very frequent. This disorder in iron homeostasis leads to an accumulation of this element in cells and secretions, including saliva. Therefore, assuming a greater susceptibility of pregnant women to BS, it was considered that a treatment with Forhans Gengi-For® would be particularly effective both in the treatment of anemia and BS.

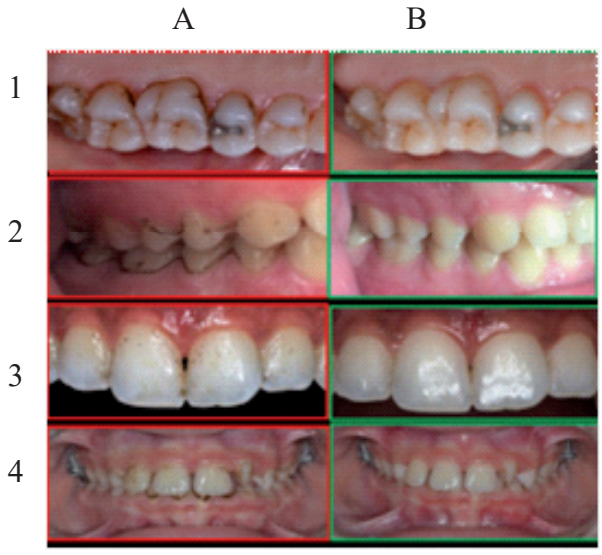

Fig. 2. Effect on Black Stain after 90 days of treatment with Forhans Gengi-For® (B) in 4 patients previously treated with a professional hygiene session (A) in accordance with the new protocol described above.

Illustrated below is the clinical case concerning a 43-year-old woman suffering from BS, which was then eliminated after a professional hygiene treatment. In the spring of 2017, during a second pregnancy, BS reoccured in the patient (Figure 3). Interestingly, the patient history showed an alteration of iron homeostasis associated with pregnancy itself, as shown by the hemoglobin values of 10.80 g / dl and ferritin of 12.95 ng / ml. After 30 days of oral treatment with 100 mg of bLf twice a day on an empty stomach, hemoglobin increased to 11.50 g / dl and ferritin increased to 14.65 ng / ml while black pigmentations tended to decrease in the absence of professional hygiene treatment. After 6 and 12 months of treatment with Forhans Gengi-For®, the patient no longer showed any signs of BS as shown in Fig. 3 and Fig. 4.

The Figure 3 shows the images related to the check-up, after 6 months of treatment with topical Forhans Gengi-For® preceded by a 30-day treatment with Lf for oral use.

The Figure 4 shows images of the check-up after 12 months of treatment with topical Forhans Gengi-For®.

Conclusions

From what has been described and from the reported cases, it appears evident that iron is the main protagonist, as well as the cause, of the formation of BS. In fact, there is strong evidence that foods fortified with iron or iron-containing vitamin complexes, enhancing the availability of this element, increase the possibility of the formation of ferric sulfide which manifests itself as a deposit of black pigments on the surface of the teeth (1). On the other hand, since every disorder of iron homeostasis leads to its overload in secretions and a lack of it in the circulation, treatment with bLf is particularly indicated during pregnancy. In fact, even if further clinical studies are required, these results indicate that Forhans Gengi-For® can be an effective and innovative therapy able to counteract this harmless but unsightly pathology through the sequestration of the excess of ferric ions in the saliva and the relative control of bacteria depending on the presence of iron for their growth.

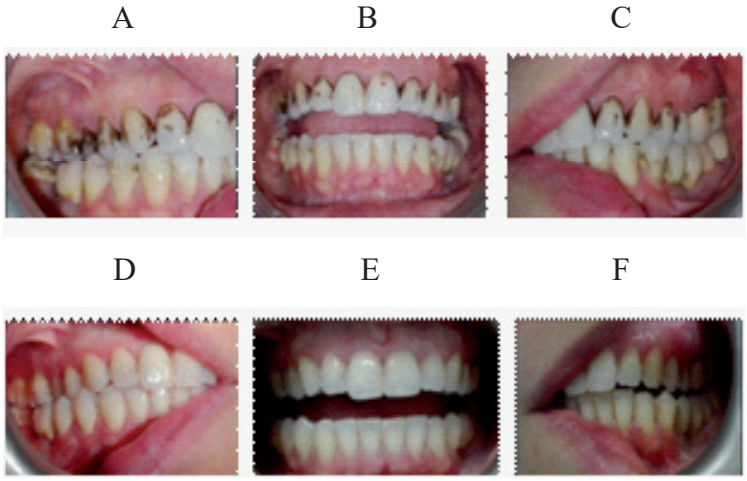

Fig. 3. Images of Black Stain before (A, B, C) and after (D, E, F) 6 months of treatment with Forhans Gengi-For®, in accordance with the described protocol.

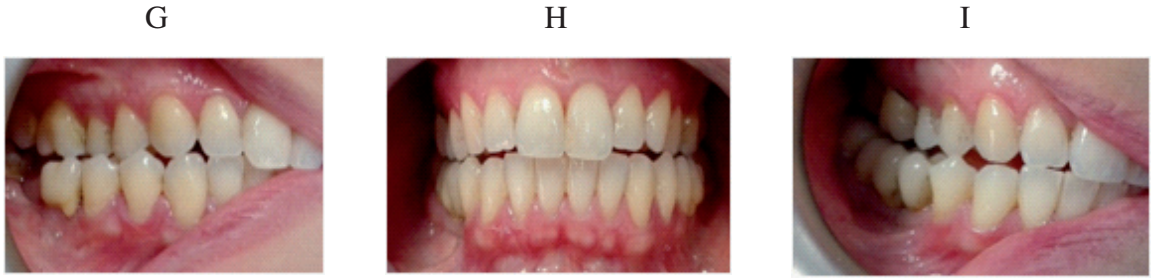

Fig. 4. Images of check-up, after 12 months of treatment with Forhans Gengi-For®

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